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(54) Title: USE OF EXONUCLEASE IN DNA SEQUENCING

(57) Abstract

Method for performing a DNA sequencing reaction in the presence of exonuclease I or exonuclease VII.

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DESCRIPTION

Use Of Exonuclease In DNA Sequencing

Background of the Invention

This invention relates to improved methods for DNA sequencing.

Tabor and Richardson, U.S. Patent No. 4,795,699, and U.S. Patent 4,962,020, describe a method for DNA sequencing using T7 DNA polymerase. Such sequencing may be performed in the presence of magnesium or manganese ions. Both patents are hereby incorporated by reference herein.

Summary of the Invention

This invention relates to an improved method for sequencing DNA in which an exogenous single-strand specific exonuclease (i.e., one with no DNA polymerase activity, and which does not degrade double-stranded DNA) is provided in the sequencing reaction. Applicant has determined that provision of a small amount (about 1 - 50 units, where one unit is the amount of enzyme which catalyzes the release of 10 nmole of acid - soluble nucleotide from denatured DNA in 30 minutes at 37 degrees Celcius under standard conditions, see U.S. Biochemicals 1990 catalog, Cleveland, OH, hereby incorporated by reference herein) of such exonuclease activity increases the sensitivity of detection of particular DNA bands within a sequencing gel.

Without being bound to any particular theory, Applicant believes that the exonuclease may improve sequencing results by degrading excess primer and/or primer molecules weakly bound to the DNA template at sites other than the primary binding site. Such weakly bound primer can also prime extensions, resulting in the production of secondary or background sequences which interfere with the interpretation of the primary sequence.

Absence of background sequence is particularly important for situations where sequence bands must be interpreted by machine or interpreted-quantitatively.

Thus, in a first aspect the invention features performing a DNA sequencing reaction in the presence of a single-strand specific exonuclease, e.g., exonuclease I, or exonuclease VII of E. coli. Such DNA sequencing reactions generally involve those reactions described by Tabor and Richardson, supra, that is, the reaction includes one or more deoxyribonucleotides and one or more dideoxyribonucleotides, a DNA polymerase, and appropriate buffer conditions. Also included in this reaction are the DNA template to be sequenced and a primer. The conditions are suitable for primer extension along the DNA template until incorporation of a chain terminating agent, such as the dideoxyribonucleotides.

In a related aspect, the invention features a kit for use in DNA sequencing which includes reagents necessary for DNA sequencing such as dideoxyribonucleotides, deoxyribonucleotides, DNA primers and the necessary buffers, and an exonuclease lacking DNA polymerase activity, as described above.

This invention not only provides the advantage of preventing primers from giving ambiguous sequences, but also causes removal of excess primer after the initiation of a sequencing reaction. This removes the primer peak in a gel and allows sequence information to be obtained from the first few bases on the template immediately adjacent the primer binding site. Thus, an extra 6 - 10 bases can be read in such an experiment. This is particularly useful in automated sequencing reactions.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

The drawing will first briefly be described.

Drawing

The figure is a graphical representation of DNA sequencing data obtained in the presence or absence of exonuclease I.

Referring to the figure, sequencing reactions were run generally as described in the SEQUENASE® Dye Primer sequencing kit protocol of A.B.I and U. S. Biochemical 1992. Specifically, four annealing reactions were performed in reaction buffer (40mM MOPS pH 7.5, 50mM NaCl, 10mM $MgCl_2$, 15mM sodium isocitrate, and 5mM $MnCl_2$) and dye-labeled universal (-21) M13 primers. The A and C annealing reactions contained 0.1 pmol of M13mp18 template DNA and 1.0 pmol of primer (FAM-labeled primer for C and JOE-labeled primer for A) in a volume of 4 μl. The G and T annealing reactions contained 0.3 pmol of M13mp18 template DNA and 3.0 pmol of primer (TAMRA-labeled primer for G and ROX-labeled primer for T) in a volume of 11 μl. Thus a total of 0.8 pmole (2μg) of M13mp18 DNA was combined in four annealing reactions. Annealing was carried out by heating at 65°C for 2 min and slow cooling to <30°C (about 35 min). Then termination mix (1000M dATP, dCTP, dTTP, 7-deaza-dGTP and 3.3M of respective ddNTP) was added to each reaction vial (1μl to the A and C reactions; 2μl to the G and T reactions, respectively) making the final concentration of nucleotide in the A and C reactions 167μM dNTP and 0.55μM ddNTP. Similarly, 133 μM dNTP and 0.44 μM ddNTP was used in the G and T reactions.

Stock enzyme contained SEQUENASE® Version 2.0 DNA Polymerase (13 Units/μl) and Inorganic Pyrophosphatase (12 Units/μl). This stock was diluted 6-fold in Enzyme Dilution Buffer (10mM Tris pH 7.5, 0.1mM EDTA). Reaction vials were pre-warmed at 37°C. Then 1μl of diluted stock enzyme (2.2 units DNA polymerase) was added to each of the A and C reactions. Similarly, 2μl (4.4 units DNA

polymerase) was added to each of the G and T reactions. The mixtures were incubated for 20 min at 37°C. The four reaction mixtures were combined into one vial and 15μl of Stop/Salt solution (1M NaOAc, 20mM EDTA), and 180μl 95% ethanol added. After 15 min at -20°C, the precipitated DNA was collected by centrifugation (12,000xg 15 min), and washed twice with 70% ethanol (repeating centrifugation).

The precipitated DNA was resuspended in 5 μl 83% deionized formamide containing 8.3 mM EDTA, heated at 95°C for 2 min and applied to an 8.3 M urea, 6% polyacrylamide gel mounted in the ABI 373A sequencing instrument. Electrophoresis was conducted at a constant 35W and 42°C for 14 hours. The data were collected and analyzed using the ABI software.

Referring to section (A) of the figure, the sequence of M13mp18 DNA with no Exo I used in the reaction mix is shown. The sequence shown is approximately 200 bases from the priming site. The upper (major) sequence is the one interpreted by the instrument software. Errors in this sequence are underlined. The lower (shaded) sequence is the complement of the sequence of M13mp18 from bases 4936-4903. This sequence aligns well with the apparent sequence represented by the background signals (smaller peaks) in this experimental run. It is the result of priming by the -21 primer at a weak site of homology at base 5077. The primary sequence is the result of priming at a 100% matched site at base 6291.

Referring to section (B) of the figure, the same DNA is shown sequenced as described above, but with a total of 3 units of Exo I added at the same time as the polymerase. The A and C reactions received 0.5 units Exo I while the G and T reactions each received 1 unit. Note that there are no errors in this sequence and that background peaks are eliminated or greatly reduced in intensity. There is no identifiable secondary sequence and the accuracy of the sequence obtained is much higher.

Other embodiments are within the following claims.

Sequence Listing

(1) GENERAL INFORMATION:

(i) APPLICANT: Carl W. Fuller

(ii) TITLE OF INVENTION: USE OF EXONUCLEASE IN DNA SEQUENCING

(iii) NUMBER OF SEQUENCES: 1

(iv) CORRESPONDENCE ADDRESS:

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(E) COUNTRY: USA
(F) ZIP: 90017

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44Mb storage
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: IBM MS-DOS (Version 5.0)
(D) SOFTWARE: WordPerfect (Version 5.1)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: 08/039,982
(B) FILING DATE: March 30, 1993
(C) CLASSIFICATION: 435

(vii) PRIOR APPLICATION DATA:

Prior applications total, including application described below: None

(A) APPLICATION NUMBER:
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Warburg, Richard J.
(B) REGISTRATION NUMBER: 32,327
(C) REFERENCE/DOCKET NUMBER: 200/013

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(C) TELEX: 67-3510

(2) INFORMATION FOR SEQ ID NO.: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GTGAGCTAAC TCACATTAAT TGCGTTGCGC TCACT 35

Claims

1. Method for performing a DNA sequencing reaction in the presence of a single-strand specific exonuclease lacking DNA polymerase activity, comprising the steps of:

contacting a DNA template and primer with a DNA polymerase, at least one deoxyribonucleotide, a chain terminating agent, and said exonuclease under primer extension conditions.

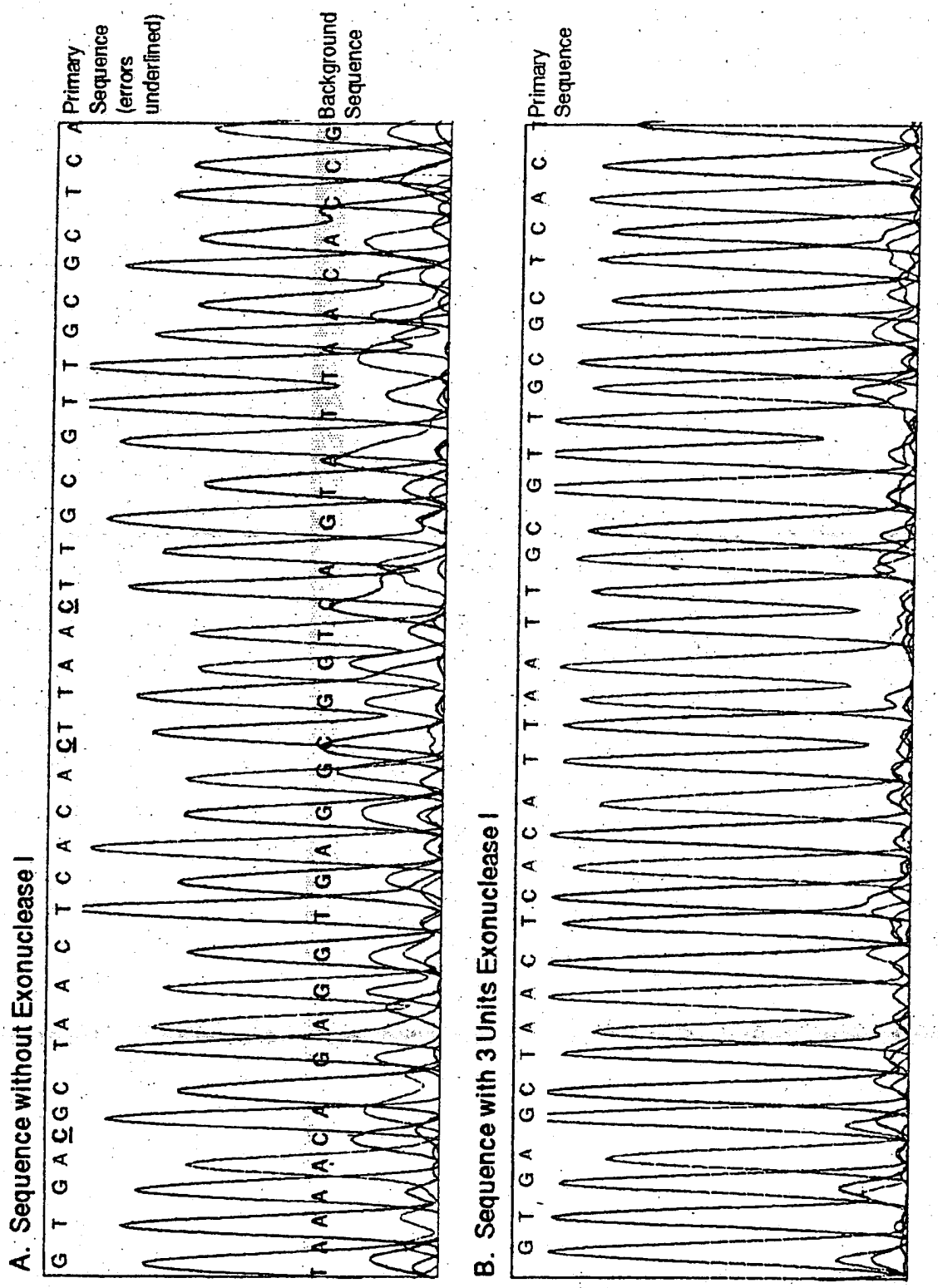
2. Kit for use in DNA sequencing comprising in separate containers one or more reagents necessary for DNA sequencing including a dideoxyribonucleotide, and a single-strand specific exonuclease lacking DNA polymerase activity.

3. The kit of claim 2, wherein the exonuclease is exonuclease I.

4. The method of claim 1 wherein said exonuclease is exonuclease I.

5. The method of claim 1 wherein said exonuclease is exonuclease VII.

6. The kit of claim 2 wherein said exonuclease is exonuclease VII.



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03416

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12Q 1/68; C07K 13/00, 15/00; C07H 19/00
US CL :435/6; 530/350; 536/22.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6; 530/350; 536/22.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, CA, MEDLINE, BIOSIS, WPI
search terms: sequencing, single-strand exonuclease, polymerase, exonuclease I, exonuclease VII

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
Y	Stratagene Catalog, issued 1988, "Gene Characterization Kits", page 39.	2, 3
Y	US, A, 4,795,699 (TABOR ET AL.) 03 January 1989, column 5, lines 17-60.	1-6
Y	Journal of Biological Chemistry, Volume 253, No. 2, issued 25 January 1978, K. R. Thomas et al, "Processivity of DNA Exonucleases", pages 424-429, especially page 428, third full paragraph.	1-4
Y	Journal of Biological Chemistry, Volume 249, No. 14, issued 25 July 1974, J. W. Chase et al, "Exonuclease VII of Escherichia coli. Purification and Properties", pages 4545-4552, especially page 4549, paragraph bridging columns 1 and 2.	1, 2, 5, 6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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